REMARKS

Status of the claims

Claims 57, 68-71, 87-91, 93 and 96-102 were pending. Claims 91, 93, and 96-102 have been withdrawn from consideration, but as they contain all of the limitations of the elected composition claims, they are eligible for rejoinder upon allowance of the claims under consideration.

Claim 57 has been amended as shown above to clarify the claimed subject matter. As the new matter rejections appear to be based on the assertion that a "non-naturally occurring zinc finger protein" necessarily means a zinc finger protein that has a non-naturally occurring overall structure and/or includes non-naturally occurring amino acids, claim 57 has been amended to specify that it is only the recognition helix region of the zinc finger domains that are non-naturally occurring. As this amendment does not change the scope of the claims (and therefore does not require a new search), entry thereof after final is in order such that the claims are pending as shown above.

Objections/Rejections Withdrawn

The objections to claims 57 and 68-71 have been withdrawn. (Final Office Action, page 11). In addition, the rejection of claims 57 and 68-71 under 35 U.S.C. § 112, 2nd paragraph as allegedly unclear has also been withdrawn. (Final Office Action, page 12). Finally, the previous rejections under 35 U.S.C. § 103(a) over various co-owned patents were withdrawn pursuant to 35 U.S.C. § 103(c)(1). (Final Office Action, pages 13-14).

35 U.S.C. § 112, 1st paragraph, written description (new matter)

Claims 57 and 68-71 were again rejected under 35 U.S.C. § 112, 1st paragraph as allegedly containing new matter not described in the originally filed specification, namely a zinc finger protein comprising at least one zinc finger domain with a non-naturally occurring recognition helix. (Office Action, pages 11-13). In addition, claims 57 and 68-71 were newly rejected under 35 U.S.C. § 112, 1st paragraph as allegedly containing new matter for reciting "non-naturally occurring." (Final Office Action, page 14-15).

These rejections appear to be based on a misunderstanding of the claimed subject matter (Final Office Action, page 12 and page 15):

> Claims 57 and 68-71 are complexes in cells in which a generic zinc finger comprising at least 3 fingers, one of which recognizes a generic non-naturally finger is bound to cellular chromatin in a region sensitive to DNAseI digestion.
>
> The specification teaches generally to mutate known domains, but does not teach the structure of all zinc finger proteins, nor does it teach what mutations are non-naturally occurring. Still further, the original claims and the original specification fail to demonstrate any possession of the genera as claimed. At best, non-naturally occurring amino acids are claimed to be an inclusive genera in the overall genera of the invention...however such fails to evince that Applicant possessed only those with non-naturally occurring amino acids, as a genera, much less the larger genera of any non-naturally occurring zinc finger protein.

However, the claims are not directed to zinc finger proteins with at least 3 fingers in which one of the fingers "recognizes a generic non-naturally finger is bound to cellular chromatin in a region sensitive to digestion with DNAseI." (Final Office Action, page 12). Nor are the claims drawn to non-naturally occurring amino acids, to unknown zinc finger proteins or to zinc finger proteins that have naturally occurring recognition helix regions. Rather, the claims are drawn to zinc finger proteins in which all the fingers are non-naturally occurring in that the 7 amino acids of the recognition helix region are designed and/or selected. For the reasons of record and reiterated herein, the **claimed** subject matter is fully described in the as-filed specification and no new matter has been added to the claims by amendment.

To reiterate, the proscription against the introduction of new matter in a patent application (35 U.S.C. §§ 132 and 251) serves to prevent an applicant from adding information that goes beyond the subject matter originally filed. *See, e.g., In re Rasmussen*, 650 F.2d 1212, 1214, 211 USPQ 323, 326 (CCPA 1981) and MPEP § 2163.06. However, literal description of claimed subject matter is never required (M.P.E.P. § 2163.02):

> The subject matter of the claim need not be described literally (i.e., using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement.

Thus, the written description requirement is satisfied if the specification reasonably conveys possession of the invention to one skilled in the art. *See, e.g., In re Lukach*, 169 USPQ 795, 796 (CCPA 1971).

Second, the disclosure must be read in light of the knowledge possessed by the skilled artisan at the time of filing, for example as established by reference to patents and publications available to the public prior to the filing date of the application. *See, e.g., In re Lange*, 209 USPQ 288 (CCPA 1981). Not only must the disclosure be read in light of the knowledge possessed by one of skill in the art, but the burden is on the Examiner to provide evidence as to why a skilled artisan would not have recognized that the applicant was in possession of claimed invention at the time of filing. *Vas Cath, Inc. v. Mahurkar*, 19 USPQ2d 1111 (Fed. Cir. 1991); *In re Wertheim*, 191 USPQ 90 (CCPA 1976).

Likewise, definiteness of claims (including the term "non-naturally occurring") must be analyzed, not in a vacuum, but in light of (1) the content of the particular application disclosure, (2) the teachings of the prior art and (3) claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. *See, e.g., Energizer Holdings, Inc. v. ITC,* 77 USPQ2d 1625 (Fed. Cir. 2006). In other words, the terms at issue must be read in context of the application and field of endeavor.

Applying this well-settled law to the instant case, it is clear that the specification as filed conveys that Applicants were in possession of the claimed subject matter, at the time of filing. With regard to a zinc finger protein comprising at least 3 fingers, each with a non-naturally occurring recognition helix, it is clear that a designed and/or selected zinc finger protein as described in detail and exemplified in the specification is inevitably a zinc finger protein whose fingers comprise non-naturally occurring recognition helices (page 5, lines 14-25 (paragraph [0019] pf published application); page 6, lines 20-31 (paragraph [0025] of the published application); page 17, lines 4-20 (paragraph [0070] of published application); Example 8 (paragraphs [0110], [0111] and [0114] of published application); Table 1 which shows exemplary non-naturally occurring zinc finger

recognition domains; Example 15 (paragraph [0158] of published application), emphasis added):

> In another embodiment, an accessible region is identified within a region of interest and a ZFP target site is located within the accessible region. <u>A ZFP that binds to the target site is designed</u>. The designed ZFP can be introduced into the cell, or a nucleic acid encoding the designed ZFP can be designed and the designed nucleic acid can be introduced into the cell, where it will express the designed ZFP. <u>Methods for the design and/or selection of ZFPs that bind specific sequences are disclosed in</u> U.S. Pat. No. 5,789,538; U.S. Pat. No. 6,007,408; U.S. Pat. No. 6,013,453; PCT WO 95/19431; PCT WO 98/54311 co-owned PCT/US00/00388 and references cited therein; co-owned U.S. patent application Ser. No. 09/444,241, filed Nov. 19, 1999; and co-owned U.S. patent application Ser. No. 09/535,088, filed Mar. 23, 2000. Methods for selection include, but are not limited to, phage display and in vivo selection.
>
> In methods comprising introduction of an exogenous molecule into a cell and testing for binding of the exogenous molecule to a binding site, <u>a ZFP that binds to a target site, located within an accessible region, is designed.</u> The designed ZFP can be introduced into the cell, or a nucleic acid encoding the designed ZFP can be designed and the designed nucleic acid can be introduced into the cell, where it will express the designed ZFP. Methods for the design and/or selection of ZFPs that bind specific sequences are disclosed in U.S. Pat. No. 5,789,538; U.S. Pat. No. 6,007,408; U.S. Pat. No. 6,013,453; PCT WO 95/19431; PCT WO 98/54311 co-owned PCT/US00/00388 and references cited therein; co-owned U.S. patent application Ser. No. 09/444,241, filed Nov. 19, 1999; and co-owned U.S. patent application Ser. No. 09/535,088, filed Mar. 23, 2000. Methods for selection include, but are not limited to, phage display and in vivo selection.
>
> In a preferred embodiment, an exogenous molecule is a zinc finger DNA-binding protein (ZFP). Certain ZFPs, their properties and their binding sequences are known in the art, as described supra. <u>Furthermore, it is possible, for any particular nucleotide sequence, to design and/or select one or more ZFPs capable of binding to that sequence and to</u>

characterize the affinity and specificity of binding. See, for example, U.S. Pat. No. 5,789,538; U.S. Pat. No. 6,007,408; U.S. Pat. No. 6,013,453; PCT WO 95/19431; PCT WO 98/54311 co-owned PCT/US00/00388 and references cited therein; co-owned U.S. patent application Ser. No. 09/444,241, filed Nov. 19, 1999; and co-owned U.S. patent application Ser. No. 09/535,088, filed Mar. 23, 2000. Certain sequences, such as those that are G-rich, are preferred as ZFP binding sites. Since a **three**-finger ZFP generally binds to a 9- or 10-nucleotide target site, in a preferred embodiment, an accessible region, present within a region of interest in cellular chromatin, is searched for one or more G-rich sequences of 9-10 nucleotides and, for each sequence so detected, a ZFP can be designed to bind those sequences. In addition, two three finger modules can be joined, via an appropriate linker domain, to form a six-finger protein capable of recognizing an 18-20 nucleotide target site. See, for example, PCT/US99/04441.

Plasmids were constructed to encode transcriptional effector proteins containing zinc finger domains designed to recognize target sites surrounding the transcriptional initiation site of the human vascular endothelial growth factor (VEGF) gene; i.e. within the +1 accessible region described in Example 7. The target site has the sequence 5'-GGGGAGGATCGCGGAGGCTT-3'(SEQ ID NO: 1), where the underlined T residue represents the major transcriptional startsite for the VEGF gene. A binding domain containing six zinc fingers, named VEGF 3a/1, was designed to bind to this 20-nucleotide target sequence. A **three-finger zinc finger domain**, VEGF 1 was designed to bind to the upstream 10-nucleotides of this target site having the sequence 5'-GGGGAGGATC-3' (SEQ ID NO: 2). A control six-fmger domain, GATA 15.5, which was designed to bind the sequence 5'-GAGTGTGTGAACTGCGGGGCAA-3' (SEQ ID NO: 3), was also used. These zinc finger domains were encoded as fusion proteins in the NVF vector, as described below.

The zinc finger domains were constructed in a SP1 backbone. **The sequences of the recognition helices**, from position -1 to position +6, of VEGF 3a/1, VEGF 1 and GATA 15.5 are shown in Table 1. ...

> The zinc finger domains contained **designed recognition helices**, as shown in Table 1, in a SP1 backbone.
>
> An engineered fusion protein was designed to recognize a unique 9 base pair sequence in the DNase I hypersensitive region at –2 kb. This protein (BOS 3) comprised a nuclear localization sequence, a zinc finger binding domain, a KRAB repression domain and a FLAG epitope. The zinc finger binding domain was targeted to the sequence GGGGAGGAG, (SEQ ID NO: 27) which is complementary to the sequence CTCCTCCCC (SEQ ID NO: 28) in the coding strand. **Zinc finger sequences (for amino acids –1 through +6 of the recognition helices)** were RSDNLTR (SEQ ID NO: 29), RSDNLTR (SEQ ID NO: 30) and RSDALTK (SEQ ID NO: 31). Construction of a plasmid encoding the fusion protein and determination of the binding affinity of the zinc finger binding domain for its target sequence were performed according to methods disclosed in co-owned PCT WO 00/41566 and WO 00/42219. The dissociation constant (Kd) was determined to be 3.5 pM.

Indeed, the well-understood definition of "non-naturally occurring" was recently addressed by the Board of Patent Appeals and Interferences, where the Board confirmed that that even when the phrase "naturally occurring" does not appear verbatim in the specification, it would clearly be understood by the skilled artisan to mean something that exists or is found in nature. See, *Ex parte Dewis* (Appeal 2007-1610, decided September 4, 2007). Plainly, the skilled artisan would have no doubt as to the scope of the term "non-naturally occurring," namely to zinc finger proteins whose recognition helices are designed and/or selection and are not found in nature.

Thus, contrary to the Examiner's assertions, the evidence of record clearly establishes that, at the time of filing, the genus of non-naturally occurring zinc finger proteins was known (recognition helix modifications, backbone, cloning, functionality, etc.). The skilled artisan would know that a non-naturally occurring recognition helix (of 7 amino acids) could readily be inserted into any zinc finger backbone to bind to a selected target site.

Moreover, as evidenced by the number of references cited in the specification regarding design and/selection of zinc finger proteins, the state of the art at the time of filing clearly evidences that the skilled artisan would understand that a zinc finger proteins can be designed to bind to regions of cellular chromatin that are sensitive to digestion with DNAseI. The whole point of the entire specification is to describe binding of molecules (e.g., non-naturally occurring zinc finger proteins) to accessible regions of cellular chromatin. As clearly described and would be evident to the skilled artisan, one way accessible regions are identified is by digestions with DNAseI (page 4, lines 13-20 (paragraph [0015] of the published application); page 13, lines 20-30 (paragraph [0057] of the published application); page 15, lines 14-19 (paragraph [0063] of the published application); Examples 2, 5, 6 and 15; and the Figures, emphasis added):

> Accessible regions are determined, for example, by identifying regions in cellular chromatin that are hypersensitive to the action of various structural probes, either chemical or enzymatic. In a preferred embodiment, an enzymatic probe is used. <u>In a more preferred embodiment, the enzymatic probe is deoxyribonuclease I (**DNase I**).</u>
>
> In one embodiment, an enzymatic probe of chromatin structure is used to identify an accessible region<u>. In a preferred embodiment, the enzymatic probe is **DNase I** (pancreatic deoxyribonuclease)</u>. Regions of cellular chromatin that exhibit enhanced sensitivity to digestion by DNase I, compared to bulk chromatin (i.e., DNase-hypersensitive sites) are more likely to have a structure that is favorable to the binding of an exogenous molecule, since the nucleosomal structure of bulk chromatin is generally less conducive to binding of an exogenous molecule. Furthermore, DNase-hypersensitive regions of chromatin often contain DNA sequences involved in the regulation of gene expression. Thus, binding of an exogenous molecule to a **DNase-hypersensitive** chromatin region is more likely to have an effect on gene regulation.
>
> In general, target sites for newly-discovered transcription factors, as well as other types of exogenous molecule, can be determined by methods that are well-known to those of skill in the art such as, for example, electrophoretic mobility shift assay, exonuclease protection, DNase footprinting, chemical footprinting and/or direct nucleotide sequence determination of a binding site. See, for example, Ausubel et al., supra, Chapter 12.

Thus, Applicants were also clearly in possession of complexes as claimed in which the zinc finger protein with the non-naturally occurring recognition helices is bound to a site in cellular chromatin that is sensitive to DNAse digestion.

Accordingly, for at least these reasons, Applicants submit that the no new matter has been added and withdrawal of the rejections is in order.

35 U.S.C. § 102

Claims 57, 68, 70 and 71 were rejected under 35 U.S.C. § 102(e) as allegedly anticipated by U.S. Patent No. 6,013,453 (hereinafter "Choo"), which was alleged to disclose the making of a mutant 3-fingered zinc finger protein that binds to the "coding sequence for a specific ras mutation" in human cells. (Final Office Action, page 16). It was also alleged t hat "absent reason to believe otherwise, this site occurs within the broad definition of a general region which is in some way sensitive to digestion with DNAseI." *Id.*

Applicants traverse the rejection and supporting remarks.

The Examiner errs in asserting that Choo teaches that their zinc finger protein binds to a site in cellular chromatin, let alone that the in vitro sequence bound by their protein is necessarily an accessible region in cellular chromatin. Rather, Example 5 (not Example 4 as cited in the Office Action), teaches that experiments in cells have yet to be done and, moreover that the planned prophetic experiments proposed by Choo do not involve cellular chromatin, but, rather plasmids (Example 5 of Choo, emphasis added):

> Following selection, a number of separate clones were isolated and phage produced from these were screened by ELISA for binding to the G12V ras sequence and discrimination against the wild-type ras sequence. A number of clones were able to do this, and sequencing of phage DNA later revealed that these fell into two categories, one of which had the amino acid Asn at the +3 randomised position, and another which had two other undesirable mutations.
>
> Assay of the protein in eukaryotes (e.g. to drive CAT reporter production) requires the use of a weak promoter. When expression of the anti-RAS (G12V) protein is strong, the peptide presumably binds to the wild-type ras allele (which is required) leading to cell death. For this reason, a

> regulatable promoter (e.g. for tetracycline) will be used to deliver the protein in therapeutic applications, so that the intracellular concentration of the protein exceeds the Kd for the G12V point mutated gene but not the Kd for the wild-type allele. Since the G12V mutation is a naturally occurring genomic mutation (not only a cDNA mutation as was the p190 bcr-abl) human cell lines and other animal models can be used in research.

This is not a demonstration about engineered zinc finger proteins forming complexes with cellular chromatin. Indeed, evidence of record establishes that the skilled artisan had absolutely no expectation cellular chromatin could be complexed with an engineered zinc finger protein. The cited reference, Choo, clearly teaches it was **not** predictable that engineered zinc finger proteins would bind to cellular chromatin: (see, Choo, col. 28, lines 39-47, emphasis added):

> The selective stimulation of transcription indicates convincingly that highly site-specific DNA-binding can occur in vivo. However, while transient transfections assay binding plasmid DNA, the true target site for this and most other DNA-binding proteins is genomic DNA. This might well present significant problems, not least since this DNA is physically separated from the cytosol by the nuclear membrane, but also since it may be packaged within chromatin.

Choo clearly states that endogenous cellular genes are different from artificial substrates, not only due to the presence of the nuclear membrane but due to the "normal chromatin environment." In addition, Choo clearly states that their randomly integrated sequences (or their episomal reporter plasmids) are not in their normal chromatin environment.

Therefore, the evidence clearly establishes that Choo does not describe or demonstrate complexes as claimed – this reference does not describe complexes of engineered zinc finger proteins and a region of cellular chromatin that is sensitive to digestions with DNAse. As such, Choo cannot anticipate any of the pending claims

35 U.S.C. § 103

Claims 57 and 68-71 were also rejected under 35 U.S.C. § 103(a) as allegedly obvious over Choo in view of WO 00/9837755 (hereinafter "Dangl"). (Final Office

Action, pages 16-17). Choo was cited as above and Dangl was cited for teaching that zinc finger proteins function in plant cells. *Id.*

For the reasons detailed above, Choo does not describe or demonstrate the claimed complexes. To the contrary, Choo teaches that complexes of zinc finger proteins with non-naturally occurring recognition helices and cellular chromatin was entirely unpredictable. Thus, the claimed complexes are not a predictable use of the individual elements, and, as such, these claims cannot be obvious over any combination of Choo and Dangl.

Still further evidence that Choo's disclosure was not seen by the skilled artisan as providing any reasonable expectation that engineered zinc finger proteins would form complexes with DNAse-sensitive regions of cellular chromatin is also of record. Well after Choo's work, the laboratories working in the field of zinc finger proteins clearly indicated that their skilled artisans regarded modulating endogenous gene expression with an engineered zinc finger protein as an unmet challenge:

> While our early experiments have focused on the regulation of genes transiently introduced into cells, we realized that the willful and specific regulation of endogenous genes with designed transcription factors has remained an unmet challenge in biology.[1]

In a separate discussion article, a co-author of this Beerli paper also discussed the complete lack of predictability of using ZFPs to bind to endogenous genes based on studies such as Choo's (which used introduced binding sites):

> "This is the first time we've been able to show that these designed transcription factors work on real genes and real chromosomes, <u>not genes of binding sites that have been introduced into cells,</u>"....[2]

---

[1] See, Beerli et al. (2000) Proc. Natl. Acad. Sci. USA 97:1495-1500, page 1465, left column, cited on page 2, line 19 of the specification

[2] See, Borman "DNA-Binding Proteins Turn Genes On and Off." February 21, 2000, C&CEN (copy attached hereto).

Thus, the skilled artisan is on the record as stating that binding of engineered zinc finger proteins to cellular chromatin was unexpected based on Choo's disclosure.

Given the clear teaching away from binding engineered zinc finger to cellular chromatin by Choo, there is no combination of Choo and Dangl that render any of the pending claims obvious over these references. Therefore, withdrawal of the rejection is in order.

CONCLUSION

For the reasons set forth herein, allowance of the claims under consideration, and rejoinder and allowance of the withdrawn claims, are requested.

Respectfully submitted,

Date: June 10, 2009

By: D Pasternak

Dahna S. Pasternak
Registration No. 41,411
Attorney for Applicants

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94303
Telephone: (650) 493-3400
Facsimile: (650) 493-3440